Claims 1-42 are rejected under 35 U.S.C.§103(a) for alleged obviousness over U.S. Patent No. 5,705,621 to Ravikumar ("Ravikumar") in view of U.S. Patent No. 4,973,679 to Caruthers et al. ("Caruthers") and further in view of U.S. Patent No. 5,548,076 to Froehler et al. ("Froehler") and further in view of Sproat et al. (PTO-892 Ref. W), Conway, et al. (PTO-892 Ref. Y), Atkinson et al. (PTO-892 Ref. Z), and Sproat et al. (PTO-892 Ref. RA). Applicants respectfully request reconsideration and withdrawal of the rejection.

The Office Action has reiterated its prior basis for it rejection. As best understood by Applicants, the Office Action asserts that the primary references (Ravikumar, Caruthers and Froehler) show a variety of solvent systems for 5'-deprotection, and do not indicate that the selection of any particular solvent system is critical. Thus, according to the Office Action, the references "motivate the selection of practically any solvent mixtures which will dissolve the reactants and not otherwise interfere with the intended synthetic transformation." Applicants respectfully disagree, and discuss each of the primary references in turn.

1. The Ravikumar Reference

The Office Action asserts that this reference (and three additional Caruthers patents therein, listed one the form PTO-892 as references I, J and K), discloses conventional oligonucleotide synthesis. The Office Action states that the Ravikumar reference describes acid-mediated 5'-hydroxyl deprotection at col. 10, lines 10-16 and col. 14, lines 5-28, but fails to provide a preferred solvent for the deprotection, and concludes from this that the reference teaches that "the choice of any particular deprotection solvent is a choice within the purview of the ordinary practitioner." Office Action at page 4. However, Applicants respectfully assert that the Office Action reads into the Ravikumar reference an assertion that does not exist. As the Office Action points out, the Ravikumar reference is a patent document which discloses the use of a novel phosphorus protecting

group for oligonucleotide synthesis. As such, the Ravikumar reference is not directed to any **particular** 5'-hydroxyl deprotection regime, and therefore does not specify any **particular** 5'-deprotection solvent for use in its methods. The Ravikumar reference's silence in this regard is intended to convey only that the Ravikumar invention will work with any solvent *that is suitable*, but does not say anything about the *scope* of solvents that *are* suitable.[1] Thus, the Ravikumar reference does not support the contention of the Office Action.

2. The Caruthers Reference

The Office Action asserts that this reference teaches at col. 5, lines 10-14 the use of "... any solvent which will dissolve the reactants ...". While the Office Action admits that "[t]he context of this statement suggests that Caruthers was making reference to the coupling step", the Office Action nevertheless asserts that "the same generic teaching appears to also apply to the deprotection step". The Office Action bases this assertion on the further assertion that the Caruthers reference discloses four deprotection reagents ($ZnBr_2$ in nitromethane; toluenesulfonic acid in chloroform:methanol; ZnBr2 in nitromethane:methanol; and 80% acetic acid), allegedly not considering any particular deprotection regime to be critical. However, Applicants respectfully assert that the Caruthers reference, read in its entirety, does not provide the broad teaching asserted by the Office Action, and in fact only teaches a narrow deprotection regime.

Applicants first note that the portion of the Caruthers reference at col. 5, lines 10-14, and cited by the Office Action, does not merely suggest, but rather explicitly refers to the coupling reaction. Contrary to the assertion of the Office Action, there is

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[1] As the Office Action points out, the Examples of the Ravikumar reference describe deprotection with dichloroacetic acid in dichloromethane. However, this fact also says nothing about the scope of deprotection solvents that are generally suitable for oligonucleotide synthesis.

nothing in the reference that suggests the use of "any solvent" for the deprotection reaction.

Further, the Caruthers reference cannot be fairly be said to teach to the skilled artisan any deprotection regime other than a Lewis acid, preferably $ZnBr_2$, in a nitromethane or nitromethane:methanol solvent. The Caruthers reference explicitly states that it's method "is predicated on color formation by triarylmethyl cation in the present of an acid, whether a Lewis acid or a protic acid." Caruthers at col. 6, lines 26-28. The Caruthers reference further states in Example 4, col. 14, lines 4-11, that when protic acids are used "3-5% depurination of each purine by protic acids is observed even when the amount of acid is reduced to the minimum amount needed to remove the dimethoxytrityl group." Indeed, the use of toluenesulfonic acid in chloroform:methanol for deprotection shown in Caruthers Table V, cited by the Office Action, is only intended to show protic acid hydrolysis rates for various trityl groups. In this regard, the Caruthers reference states at col. 15, lines 51-61:

> Several of the triarylmethylchlorides were condensed with the 5' hydroxyl of appropriately protected deoxynucleosides. These compounds are listed in Tables IV and V. The 5'-triarylmethyldeoxynucleosides were treated with protic and Lewis acids using carefully controlled conditions. The results of these studies are also recorded in Tables IV and V. These results show that several triarylmethyl groups forming different colors in acid solutions are hydrolyzed at approximately the same rapid rate in the presence of ZnBr2. **The rates are more variable -with [sic] protic acids.** (emphasis added)

The Caruthers reference teaches away from such variability in deprotection rate (and therefore the toluenesulfonic acid in chloroform:methanol cited by the action) at col. 16, lines 22-28, stating:

> [t]he various color coded triarylmethyl groups should preferably by hydrolyzed at approximately the same rate. Otherwise, each addition cycle must be individually monitored ..."

Thus, the art skilled, reading the Caruthers reference, would not understand the Caruthers reference to teach toluenesulfonic acid in chloroform:methanol, or any other protic solvent or solvent system therefor, such as is recited in the present claims.[2]

The Office Action further cites the Caruthers at col. 19, lines 47-50 as teaching deprotection with 80% acetic acid. However, that passage does not refer to the deprotection of a support-bound species having its appended protecting groups, but rather describes the 5'-terminal deprotection of a completed deoxyoligonucleotide that had already been removed from the solid support, and which also had been subject to additional deprotection to remove amino protecting groups. Further, as stated above, the Caruthers reference teaches away from the use of such protic acids. Thus, the skilled artisan would not understand the Caruthers reference to teach deprotection of a support bound oligonucleotide with 80% acetic acid.

In view of the discussion above, it can be seen that the only teaching of 5'-triarylmethyl deprotection in the Caruthers reference is the use of a Lewis acid, preferably $ZnBr_2$, in nitromethane or nitromethane:methanol solvent. There is therefore no basis for the assertion that the Caruthers reference motivates "any solvent" for deprotection.

3. The Froehler Reference

The Office Action asserts that this reference teaches a "whatever works best" philosophy, citing the Froehler reference at col. 5, lines 26-28 and lines 38-47.

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[2] Applicants assert that for this reason alone, the present claims cannot be obvious in view of the Caruthers reference.

However, as the Office Action appears to admit, the passage at col. 5, lines 26-28 reads in full:

> The solvent **for the condensation reaction** is an anhydrous organic solvent, preferably anhydrous pyridine/acetonitrile in volume proportions of 1:1. (emphasis added)

Thus, this passage explicitly refers to the condensation reaction. The Office Action further states:

> This "whatever works best" philosophy apparently also applies to the deprotection step; see column 5, lines 38-47. The last line of this portion of column 5 is particularly instructive. After listing 3 (three) different deprotection reagent/solvent mixtures, Froehler suggests a very flexible "whatever works" approach by further stating that "[o]ther deprotection procedures suitable for other known protecting groups will be apparent to the ordinary practitioner."

Office Action at page 5. However, the cited passage of Froehler recites:

> The solution containing the residual dehydrating agent and nucleoside H-phosphonate is removed from the carrier after each cycle by washing with an organic solvent such as acetonitrile. Thereafter, the protecting group is removed from the added nucleoside, preferably by treatment with a 2.5% vol/vol dichloroacetic acid/CH_2Cl_2 solution, although 1% w/v trichloroacetic acid/CH_2Cl_2 or ZnBr-saturated nitromethane also are useful. Other deprotection procedures suitable **for other known protecting groups** will be apparent to the ordinary artisan. (emphasis added)

With due respect, Applicants respectfully assert that the second sentence of the cited passage merely points out three specific deprotection reagents for use with the "triphenylmethyl ethers of the ribose or deoxyribose hydroxyl substituents" described in

Froehler.[3] Further, the last sentence, which the Office Action considers "particularly instructive", merely states that other procedures for **other** known protecting groups will be apparent to those of skill in the art, but does not allude to what these protecting groups or procedures for their removal might be. Further, the cited passage appears to suggest that different procedures are required for "other" such protecting groups. Thus, it cannot be fairly said that cited passages in Froehler supports the proposition that a variety of deprotection solvent regimes are employed such that the skilled artisan would consider the choice of any particular deprotection solvent to be routine.

In view of the discussion above, Applicants respectfully assert that the teachings of the primary references, each taken individually or taken together as a whole, and contrary to the assertion of the Office Action, do not "motivate the selection of practically any solvent mixtures which will dissolve the reactants and not otherwise interfere with the intended synthetic transformation."

The Office Action states on page 7 that the secondary references (the two cited Sproat et al. references, Conway et al. and Atkinson et al.) provide:

> ... disclosures that at least two different nucleoside 3'-O-phosphoramidites, at least one dinucleotide derivative, and some other nucleoside derivatives may be effectively dissolved in the aromatic hydrocarbon solvents benzene and/or toluene.

and further states that the disclosure of the solubility of such 3'-O-phosphoramidites, dinucleotide derivative, and "other" nucleoside derivatives provides:

> ... factually specific motivations for the ordinary practitioner conducting routine experimentation to substitute benzene, toluene, or their closely related aromatic solvent relatives as substitutes for at least a portion of the

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[3] Applicants note in this regard that Froehler exemplifies only 5'-dimethoxytrityl protecting groups.

> solvents typically used during the deprotection step in oligonucleotide synthesis."

Id. However, as discussed above, the central premise of the Office Action's argument is erroneous, because the primary references, each read as a whole, simply do not provide the motivation to substitute "any solvent that works" for the disclosed reagents.

Moreover, as apparently admitted by the Office Action, the secondary references merely provide solvents said to dissolve certain specific reagents used in certain oligonucleotide synthetic protocols, but do not disclose the use of Applicants' claimed solvents for 5'-deprotection.

The Office Action on page 9 has interpreted Applicants' prior remarks to suggest that Applicants consider the prohibition against rejections based on the "obvious to try" standard to render all routine experimentation to be "out of bounds." With respect, this is not what Applicants have asserted. Applicants asserted, and continue to assert, that where the basis of an obviousness rejection is that it would have been obvious to try the asserted combination or make the asserted modification to the prior art, *without the legally required teaching of motivation to make the combination or modification,* the rejection is improper. Where there is such motivation, and where routine experimentation would lead to the combination or modification, a rejection is proper. However, what Applicants assert herein, is that the cited art does not provide the teaching or motivation asserted by the Office Action; i.e., that the cited art does not motivate the use of any and all solvents that are capable of working in a deprotection protocol. Without that teaching or motivation, the naked possibility that one could experiment with solvents and achieve Applicants' claimed invention is a rejection based on the impermissible "obvious to try" standard.

Finally, the Office Action asserts that the present invention appears not to have solved any particular problem in oligonucleotide synthesis, or generated a basis for

unexpected results. Applicants respectfully disagree, and refer to the present specification at page 6, lines 18-27, which reads:

> The removal of trityl and other protecting groups is generally carried out in the presence of halogenated solvents such as dichloromethane or dichloroethane. However, the use of such halogenated solvents is undesirable for several reasons, particularly in relatively large scale applications such as the manufacture of oligonucleotides or analogs use as antisense agents. Consequently, there remains a need for methods of synthesis of oligonucleotides which provide improved efficiency and reduced disposal problems.

and to the specification at page 15, lines 3-12 and 30-35, which reads:

> Heretofore, deblocking of 5'-hydroxyl groups has been accomplished using such protic acids in a halogenated alkyl solvent such as dichloromethane or dichloroethane. However the use of such halogenated alkyl solvents is greatly disadvantageous because they are not easily disposed of (and therefore expensive to use) because of the environmental hazard they pose. For example, methylene chloride has been classified as a carcinogen by OSHA, and such low boiling solvents require a relatively large investment in recycling equipment.
>
> ...
>
> In some preferred embodiments, the solvent is high boiling; i.e., it has a boiling point greater than about 60°C. Such solvents are additionally advantageous in that they do not require a substantial investment in recycling equipment that meets stringent environmental regulations required by OSHA for lower boiling solvents.

It can be seen that the specification clearly sets forth the problems of using halogenated alkyl solvents in deprotection reactions, and explains why the elimination of

such reagents in the deprotection regime is desirable - e.g., that the elimination of halogenated alkyl solvents in the synthesis of oligonucleotides fulfills an important ecological end, and also eliminates the use of a known carcinogen (dichloromethane) which poses hazards to workers who handles the solvent.

In view of the discussion above, Applicants respectfully request withdrawal of this rejection under 35 U.S.C.§103(a).

Claims 1-42 are rejected under 35 U.S.C.§103(a) for alleged obviousness over Horn et al., *Nucleic Acids Research* **1989**, *17*, 6959-6967 ("Horn CB"), in view of Horn et al., *Nucleosides and Nucleotides*, **8** (5&6), 875-877 (1989) ("Horn UA").

The Horn CB reference describes the synthesis of branched oligodeoxyribonucleotides. The Horn et al. reference states that the standard deprotecting reagent was found to be ineffective for deprotection of the synthesized branched DNA, and that trityl deprotection of such branched structures was achieved using 3% DCA in toluene. Applicants wish to note that in the context of the present invention, i.e., synthesis of linear oligonucleotides, the occurrence of branched structures such as described in the Horn et al. are contaminants to be avoided, and, in the event that such branch structures are produced, it is highly desirable to avoid deprotecting them, both to eliminate participation in further synthesis cycles, and in order to utilize the trityl groups to eliminate the contaminant from the final purified linear oligonucleotide. Thus, Applicants assert that those of skill in the art would not be led to use the stringent deprotection regime disclosed in Horn et al. for standard synthesis of linear oligonucleotides.

With respect to the Horn UA reference, Applicants note as a preliminary matter that no copy of this reference has been provided, although the form PTO-892 received with the present Office Action states that a copy has been ordered. Thus, it

appears that the Office Action has levied this rejection without having seen the reference upon which it is based. Applicants respectfully assert that such a rejection is not proper. Nevertheless, Applicants have obtained a copy of the reference, which is provided herewith for the Examiner's convenience.

The Office Action asserts that according to the Horn CB reference, the Horn UA reference:

> ... discloses further details relevant to the application of dichloroacetic acid/toluene mixtures in de-tritylation of 5'-tritylated oligonucleotide precursors during single and multiple/simultaneous synthetic oligonucleotide chain extensions.

Office Action at page 10. However, the only specific reference made to the Horn UA reference (which is citation (2) of the Horn CB reference) in the Office Action is on the form PTO-892, which refers to the first full paragraph on page 6965. This passage states:

> In our early attempts to synthesize **branched DNA**, we found it difficult to deprotect the multiple intramolecular dimethoxytrityl functions with **standard DCA/CH_2Cl_2** even with extended exposure (2). Under the assumption that dimethoxytrityl stacking stabilize the protection, we employed 3% (v/v) DCA in toluene. With this solution it was possible to rapidly and efficiently detritylate the branched materials (Figure 2). (emphasis added)

Horn CB reference at p. 6965, first full paragraph. Thus, the Horn CB reference teaches 1) that **deprotection with DCA in methylene chloride is *standard***; and that 2) the deprotection referred to in the Horn UA reference is deprotection of **branched DNA**, which posed particular problems. Thus, the description of the Horn CB reference is consistent with, and indeed supports Applicants' assertion that standard deprotection regimes exist, and are not deviated from absent extraordinary circumstances, i.e., as in

deprotection of branched DNA, which is resistant to deprotection using the "standard" solvent.

With respect to the Horn UA reference itself, Applicants note that it discusses using an additional protecting group strategy for "comb" type branched DNA (removal of FMOC or levulinyl protection with t-butylamine/pyridine or hydrazine hydrate/pyridine/acetic acid), but does not refer to the application of dichloroacetic acid/toluene mixtures in de-tritylation at all. Thus, the Horn UA reference does not add to the teaching of the Horn CB reference.

In view of the discussion above, Applicants respectfully request withdrawal of this rejection under 35 U.S.C.§103(a).

Applicants believe that the claims are in condition for allowance. An early Office Action to that effect is, therefore, earnestly solicited.

Attached hereto is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "VERSION WITH MARKINGS TO SHOW CHANGES MADE."

Respectfully Submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

Claim 42 has been amended as follows.

42. (Once Amended) A method for the preparation of a linear phosphorus-linked oligomer comprising the steps of:

(a) providing a solid support;

(b) attaching a 5'-O-protected nucleoside to the solid support;

(c) deprotecting the 5'-hydroxyl of the nucleoside with a deprotecting reagent comprising dichloroacetic acid [DCA] in toluene;

(d) reacting the deprotected 5'-hydroxyl with an 5'-protected activated phosphorus compound to produce a covalent linkage therebetween;

(e) oxidizing or sulfurizing the covalent linkage to form a phosphodiester, phosphorothioate, phosphorodithioate or H-phosphonate linkage;

(f) repeating steps c through e at least once for subsequent couplings of additional activated phosphorus compounds, to produce the completed phosphorus-linked oligomer; and

(g) cleaving the oligomer from the solid support;

wherein steps (b) through (f) are performed using an automated device;

wherein said oligomer is a linear oligomer.